

Supplementary information and a commentary for consideration by the Independent Science Board and State Water Resources Control Board regarding the Delta Science Program Panel Summary Report of the Delta Outflows and Related Stressors Workshop

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In order to assist the State Water Resources Control Board (State Water Board) in updating the Bay-Delta Plan and flow objectives, the Delta Science Program convened a workshop in May 2014 on Delta Outflows and Related Stressors. A summary report (Reed et al. 2014), based on presentations, public comments, and selected literature was prepared addressing some of the key scientific uncertainties and disagreements related to low salinity habitat and flow. The report also addressed specific questions that were provided as part of their charge. The panel consisted of Drs. Denise Reed (Chair), Ernst B. Peebles (Lead Author), Paul Montagna, Kenneth Rose, Pete Smith, James T. Hollibaugh, and Josh Korman. These panel members were selected for their expertise and reputation concerning fisheries biology, hydrology, estuarine ecology, hydrodynamics, microbiology, effects of flow on fish behavior, and environmental statistics (<http://deltacouncil.ca.gov/science-program/workshop-delta-outflows-and-related-stressors>).

Among the specific questions the panel members were charged with addressing were questions associated with “other stressors”; for example, they were asked, “Can we reasonably expect that addressing other stressors without addressing flow will lead to specific improvements in the status of estuarine fish, estuarine fish habitat, and other important ecosystem attributes?” While none of the members of the panel were nutrient specialists, one approach they selected, to address this charge was to evaluate the phenomenon of ammonium (NH_4^+) inhibition of nitrate (NO_3^-) uptake and phytoplankton growth. This is, without question, a difficult and locally controversial subject, but it is a phenomenon with a well-known and complex associated biology. The panel undertook an “*in-depth consideration of this possibility*” (p. 48), and reported (p. 4), “*delta science is rapidly evolving and [...] the panel was unable to fully consider much of the review material provided in detail*”. Some of the detailed material that was not considered was data described in peer-reviewed papers that addressed this phenomenon in the Bay Delta (e.g., Wilkerson et al. 2006, Dugdale et al. 2007, 2012, 2013, Parker et al. 2012 a, b, Glibert et al. 2014 a, b). Our objectives here are to supply and summarize existing and emerging findings to aid in resolution of some of the questions the workshop panel itself posed on the topic of the role of NH_4^+ in phytoplankton dynamics, and to highlight some areas of the report that could be interpreted differently. We are unaware that there is in place any formal mechanism to supply feedback about the report.

1. Ammonium inhibition of nitrate uptake by phytoplankton

At issue is the core concept that NH_4^+ concentrations in the Bay Delta are at levels that inhibit the uptake of NO_3^- and ultimately may reduce the growth rate of phytoplankton. This hypothesis was proposed as a potentially important mechanism controlling productivity in the Bay Delta (e.g., Wilkerson et al. 2006, Dugdale et al. 2007, 2012, 2013, Parker et al. 2012 a, b). The panel recognized that NH_4^+ inhibition of NO_3^- uptake is “*not in question*” (p. 48, 49) and summarized in the highlighted box on p.48 “*There is a large body of work indicating that ammonium concentrations greater than some*

threshold inhibit the uptake of nitrate by phytoplankton". Their next statement, however, that *"high ammonium concentrations and growth on ammonium will always correlate with low phytoplankton biomass, while growth on nitrate will always correlate with high biomass accumulation, i.e., blooms"* is correct only in the case when more NO_3^- is present than NH_4^+ . We named this the ammonium paradox (Dugdale et al. 2012).

The panel continues with the statement *"If phytoplankton growth is truncated for reasons other than nitrogen limitation (e.g., light, grazing) prior to reaching "bloom" conditions, then no nitrate will be consumed and some ammonium will remain which has been interpreted (we believe incorrectly) as evidence that ammonium had inhibited bloom formation"* (p.48). This is not in conflict with our results and in fact is essentially describing the sequence of events in bloom formation (Parker et al. 2012a) where NH_4^+ concentrations must be reduced by phytoplankton uptake to levels allowing NO_3^- uptake to occur, with subsequent blooming. The causes of the elevated NH_4^+ may be direct (i.e. loading in excess of phytoplankton uptake) or indirect (e.g. water column instability and poor irradiance conditions, or low residence time; Parker et al. 2012a) and reduce the growth rate and ability of phytoplankton to drawdown NH_4^+ . Either situation (direct or indirect) will ultimately reduce or prevent the uptake of NO_3^- and so the potential for a bloom to occur.

Beneath the highlighted box in the panel report associated with the statement quoted above (p.48), the key paper cited is Cloern and Jassby (2012) which does not address $\text{NH}_4^+/\text{NO}_3^-$ /phytoplankton interactions at all. This highlighted comment also calls into question our peer-reviewed papers without any opportunity or mechanism for us to explain their misinterpretations of our observations.

In response to their highlighted box statement, the physiological regulation of NH_4^+ on NO_3^- is not in question so the physiological interpretation is that NO_3^- uptake will be inhibited under these circumstances (i.e. elevated NH_4^+) blocking access to the greater DIN pool and so preventing chlorophyll accumulation fueled by the larger NO_3^- pool until NH_4^+ is reduced. Failure of chlorophyll accumulation due to truncation of the bloom initiation sequence is only one of the possible consequences of the high NH_4^+ state; changes in community structure are also likely.

The panel also writes *"The "ammonium toxicity" paradigm, as applied to phytoplankton dynamics in northern San Francisco Bay, derives from observations primarily of the inhibition of nitrate uptake by phytoplankton in the presence of elevated ammonium concentrations"* (P.49). It should be noted that we do not use the term " NH_4^+ toxicity" with regard to the effects due to NH_4^+ inhibition of NO_3^- uptake.

2. Growth on nitrate versus ammonium

The panel asked the following important question (p. 51), *"A critical question that has not been adequately addressed is [1] whether or not phytoplankton grow "better" (faster, more efficiently) on NO_3^- than on NH_4^+ . Would elevated NH_4^+ concentrations (comparable to the concentrations of NH_4^+ plus NO_3^- currently found in the bay) support a bloom comparable in magnitude to that supported by an equivalent amount of NO_3^- , assuming bloom formation was not truncated by other factors? Related to this question is [2] the possibility that phytoplankton community composition might change in response to growth on NH_4^+ versus NO_3^- (all other things being equal), which might have implications for trophic transfer."* These questions were addressed in our recent "Nutrient Forms and Ratios" Research Project funded by the Delta Stewardship Council (<http://deltacouncil.ca.gov/scienceprogram/projects/understanding-effects-nutrient-forms-nutrient-ratios-and-light-availability>) and some of our findings were summarized in two recent peer reviewed publications that were not supplied to the panel (Glibert et al. 2014 a, b) and so were not

included in their analysis. These findings (along with our previous papers) show that many phytoplankton do grow “better” on NO_3^- and community composition may change when exposed to one N form versus another.

In multiple studies that we have conducted (Dugdale et al. 2007, Wilkerson et al. 2006, Parker et al. 2012a, b), we have routinely found that the rate of N uptake (VN) is higher for cells growing on equivalent amount of NO_3^- than for NH_4^+ (Dugdale et al. 2007, Parker et al. 2012a); that the carbon uptake (VC) or primary productivity is higher when phytoplankton access NO_3^- compared to NH_4^+ (Parker et al. 2012a, b). In Glibert et al. (2014b) when measurements of the uptake rate of N were made for enriched paired experiments conducted in the Bay Delta over multiple seasons and years, in which some samples received a NO_3^- enrichment and others an equivalent NH_4^+ enrichment, higher chlorophyll yield and higher rates of N-productivity were found in those treatments receiving NO_3^- compared to those receiving NH_4^+ (Glibert et al. 2014b). Comparable findings for the Bay Delta showing that both rates of productivity and phytoplankton community composition differ on different N forms have been reported for other sites. Low rates of productivity in the presence of elevated NH_4^+ conditions have been reported in other river, estuarine and coastal ecosystems impacted by wastewater effluent NH_4^+ (e.g. MacIsaac et al. 1979, Yoshiyama and Sharp 2006, Waiser et al. 2011, Xu et al. 2012).

The panel stated (p. 52) that, “*before decisions are made that assume NH_4^+ inhibition is occurring, the panel recommends that more information be obtained on whether the growth rate of phytoplankton is lower on NH_4^+ or NO_3^- at the concentration typically encountered in San Francisco Bay. These experiments should also examine selection for phytoplankton community composition by these two different N sources.*” Apparently the panel was unaware of our findings and project funded by the Delta Stewardship Council and other sources.

Regarding their second question, the composition of the phytoplankton that ultimately thrives under enriched NH_4^+ vs NO_3^- conditions may vary. While metabolic pathways in all cells are fundamentally similar, there are large differences in metabolism in different types of algae, and this leads to different winners and losers under different nutrient conditions. Our experiments in the Bay Delta confirm that diatoms increased at nearly twice the rate under NO_3^- enrichment as with NH_4^+ enrichment, but cyanobacteria increased in treatments with NH_4^+ particularly under low light conditions (Glibert et al. 2014b). Since these Bay Delta experiments were done under conditions in which large grazers were removed, such changes in phytoplankton composition cannot be interpreted merely in the context of larger cells being more readily eaten. Comparable results have been reported in a range of other mesocosm and field studies. In a series of mesocosm studies conducted in Wascana Lake, Canada, it was found that with enrichment with NO_3^- the proportional increase in chlorophyll was higher than with NH_4^+ enrichment and diatom biomass increased much faster in the NO_3^- enriched systems, while that of different phytoplankton - cyanobacteria and chrysophytes - increased and remained higher in the NH_4^+ treatments (Donald et al. 2013). Moving toward estuaries and coastal systems, numerous examples also exist of differential phytoplankton production based on the quality of the N load. Both Glibert et al. (2004) and Heil et al. (2007) showed that phytoplankton community composition in Florida Bay and on the West Florida Shelf was related to N form, with diatoms associated with proportionately more NO_3^- and cyanobacteria and dinoflagellates associated with reduced forms of N.

3. Effects of other factors

In their report, the panel questioned whether field observations which infer preferential uptake of NO_3^- by large cells, may simply be the effect of other factors (grazing, light limitation; p. 52). Again, there is

considerable support in the literature that cell size is related to N speciation. Stolte and Riegman (1995), in their classic paper on this topic, state, “It is concluded that NO_3^- uptake is more related to phytoplankton cell size than is NH_4^+ uptake...large algae are better competitors for NO_3^- uptake under fluctuating conditions because of larger storage volume”. They also cite a wide range of field studies in which this size difference is substantiated. In studies in the Gulf of Riga, Baltic Sea, most of the reduced forms of N were found to be taken up by the small size fraction of the phytoplankton, while NO_3^- was taken up by cells $> 5 \mu\text{m}$ in size (Berg et al. 2003). Thus, both cell size and composition change with nutrient sources.

4. Effect of light on primary productivity and producers

Regarding how light affects phytoplankton, the panel members seem to be on both sides of the fence, so to speak. On the one hand, they strongly state the dogma that phytoplankton productivity is light limited due to high sediment loads. On the other hand, they recognize that light is increasing and even argue that microphytobenthos (benthic algae) may be more important than previously thought in contributing to productivity. Additionally light will affect nitrogen metabolism. Whereas uptake of NO_3^- is generally light-dependent, that of NH_4^+ is less so. This has important implications for which type of phytoplankton thrives under different light regimes.

Moreover, with potentially increasing abundance of flagellates in the food web, the role of mixotrophy (i.e. obtaining food from both photosynthesis and grazing) must be considered. Virtually all flagellates are mixotrophs (e.g., Flynn et al. 2013) and have better capability to survive and even proliferate under low light conditions, compared to other algae. Diatoms are the only group that has not been found to have mixotrophic properties; they are true autotrophs (use photosynthesis only) and thus their light dependence is greater. A more reducing environment (more NH_4^+ relative to NO_3^-) is going to favor mixotrophs by virtue of their nutritional physiology, and a low light environment will also be in their favor as they can be less dependent on photosynthesis if they have an alternate nutritional source. Many flagellates have lower rates of growth than diatoms and thus overall productivity rates are depressed for fundamental physiological reasons - and these reasons cannot be ignored even when other factors are considered.

5. Acceleration in nitrate uptake

The panel described the geochemical model of Dugdale et al. (2013) in which productivity of Suisun Bay was modeled using terms for time-varying rates of maximal NO_3^- uptake as a function of NO_3^- concentration and a term for inhibition of NO_3^- uptake by NH_4^+ . The “time varying rate” for NO_3^- uptake includes an “acceleration factor” representing the up-regulation of the cellular machinery to process NO_3^- in response to its availability. That enzymes or other metabolic processes are up-regulated in response to additional substrate is a known biological process. This “acceleration” in uptake was first described and derived in a series of enclosures filled with coastally upwelled water (Wilkerson and Dugdale 1987) and modeled by Zimmerman et al. (1987) and Dugdale et al. (1990) and was explained in depth in Dugdale et al. (2013, pp. 292-296).

The panel stated (p. 52) that the “*acceleration factor for NO_3^- uptake was derived through a sensitivity analysis to fit model data to mesocosm data*” This is misleading and suggests that it is merely a factor to make the model run better. The panel recommended that such time-varying rates be “*estimated from experimental data*”. In fact, the acceleration term used in Dugdale et al. (2013) was calculated from data of measured NO_3^- uptake rates in mesocosm experiments in SF Bay and not based on model fits, and it

shows quite clearly that the time to draw down all available NO_3^- was invariant – this result requires that the rate of N uptake (VN) must vary with the initial NO_3^- available.

6. Analysis of Figure 7, (p. 51)

The panel presented a figure (*their Figure 7*) to offer an illustrative interpretation showing a mass balance relationship between nitrogen (N) consumption and chlorophyll production, assuming constant growth. They suggested that if NH_4^+ were drawn down first (due to preferential uptake) during the period in which biomass is low, then more biomass accumulation would occur when the only remaining N substrate available, NO_3^- , supported growth. While their simplistic illustration does show that more accumulation would occur on NO_3^- at the latter stages of exponential growth, a fact we have no disagreement with, the overall relationship presented does not adequately reflect phytoplankton biology.

First, the conversion factors they used (and giving them the benefit of the doubt for units for carbon that were not provided) are not typical compared to those found in the literature on chlorophyll yield per unit N consumed (e.g., Edwards et al. 2005, Gowen et al. 1992). With more realistic conversion factors, the chlorophyll accumulation illustrated in their Figure 7 would require $>100 \mu\text{M-N}$. Also there is an assumption that a single growth rate, μ , applies at both phases of the curve, i.e. for both growth on NH_4^+ and on NO_3^- . This figure should show a slower rate of growth on NH_4^+ and an increase in growth as the cells gain access to the NO_3^- when inhibition is removed and show accelerated uptake. All N forms are not used equally (except under controlled conditions of steady state growth on single forms of N) and phytoplankton physiology reflects this fact.

In order for their Figure 7 to be valid, no change in carbon uptake (VC) should be seen in enrichment mesocosms. This is not the case; rather, VC has been shown to vary with VN, which is higher when phytoplankton grow on NO_3^- (Parker et al. 2012a) (Note, these data were not included by the panel, who cited only a powerpoint slide from Parker's Ph.D Thesis to describe VC vs VN, in the report).

Additionally, the importance of flow on growth relationships was not considered. Not only does the rate of uptake and growth change with different N forms, but in a fluid environment both nutrients and chlorophyll can be exported downstream. In Dugdale et al. (2012), describing the natural conditions in the northern estuary, inputs of both NO_3^- and NH_4^+ occur in a flowing system with NH_4^+ concentrations sufficiently high to inhibit NO_3^- uptake upstream. Chlorophyll remains low as growth is supported by NH_4^+ (slower growth rate and generally smaller cells). The maximum biomass produced is limited by the NH_4^+ input while the larger NO_3^- pool is exported downstream, ultimately reaching the coastal ocean depending on the rate of flow and other environmental factors. With less flow, there is time for the NO_3^- pool to be accessed and chlorophyll to accumulate. The current drought conditions of 2014 presented a natural test of this hypothesis. With less flow and longer residence time, spring blooms occurred in Suisun Bay in 2014 (Glibert et al. 2014a).

Summary

In sum, the conclusions of the panel that NH_4^+ is inconsequential in this system and that only other factors (light, residence time, grazing) are important are incomplete as additional phytoplankton physiological literature and data collected in Bay Delta were not considered, and published “inhibition observations” were misinterpreted. Fundamental biological processes cannot or should not be dismissed by the panel or by the State Water Board.

We agree on several points made by the panel. We agree that flow plays a critical role in the ecology of the estuary and that it is unclear at present exactly how flow interacts to improve conditions for species of concern (or influences beneficial uses). We also agree that flow affects the nutrient field in a variety of ways including dilution of load to reduce concentration and increased residence time (allowing for microbial biogeochemical processing of nutrient pools, which alter N:P and $\text{NH}_4^+:\text{NO}_3^-$). Also in agreement with the panel, we support their recommendation that more modeling should be carried out, especially coupled models. Specifically, these models should include different nutrients, forms and nutrient ratios in addition to the phytoplankton and grazers proposed by the panel, since many models currently in use in the Bay Delta do not include nutrients, for example, the model in Lucas and Thompson (2012), cited as a key paper on p.53. Finally, we agree that much can be learned from comparative ecosystem studies around the world. It is unfortunate that the panel was not familiar with the deeper global literature on nutrient physiological ecology, and were not supplied with recent publications on these topics from the Bay Delta.

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